PATENT COOPERATION TREATY

PCT Rec'd PCT/PTO 25 MAY 2005

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PO45554PCT MVE	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/NL 03/00831	International filing date (day/month/year) 25.11.2003	Priority date (day/month/year) 25.11.2002
International Patent Classification (IPC) or both national classification and IPC C12N5/06		
Applicant ACADEMISCH ZIEKENHUIS BIJ DE UNIVERSITEIT ...et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
23.06.2004	13.04.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Lanzrein, M Telephone No. +49 89 2399-7358

Form PCT/IPEA/409 (Cover Sheet) (January 2004)

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/NL 03/00831

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-50 as originally filed

Claims, Numbers

1-28 as originally filed

Drawings, Sheets

1/8-8/8 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT International application No. PCT/NL 03/00831

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees, the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-16, 25-28
	No:	Claims	17-24
Inventive step (IS)	Yes:	Claims	1-16, 25-28
	No:	Claims	17-24
Industrial applicability (IA)	Yes:	Claims	1-28
	No:	Claims	

2. Citations and explanations

   see separate sheet

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/NL 03/00831

Reference is made to the following documents:

D1: WO 01/98456 A (JAPAN TOBACCO INC ;GETZENBERG ROBERT H (US); GENE LOGIC INC (US);) 27 December 2001 (2001-12-27)

D2: KILE BENJAMIN T ET AL: "Functional analysis of Asb-1 using genetic modification in mice." MOLECULAR AND CELLULAR BIOLOGY, vol. 21, no. 18, September 2001 (2001-09), pages 6189-6197, XP002238792 ISSN: 0270-7306

D3: KILE B T ET AL: "Cloning and characterization of the genes encoding the ankyrin repeat and SOCS box-containing proteins Asb-1, Asb-2, Asb-3 and Asb-4" GENE, ELSEVIER BIOMEDICAL PRESS. AMSTERDAM, NL, vol. 258, no. 1-2, 27 November 2000 (2000-11-27), pages 31-41.

Re Item IV

Lack of unity of invention

This Authority considers that there are 4 inventions covered by the claims indicated as follows:

1) Claims 1-16: Method for in vitro expansion of mammalian cells or progenitor cells utilizing an Asb-a polypeptide, fusion proteins thereof or nucleic acid encoding said polypeptide.

2) Claims 17-25 (partially): An Asb-a polypeptide having an amino acid sequence with at least 39 % amino acid identity with SEQ ID NO: 1, nucleic acid thereof having at least 35% identity with a nucleotide sequence depicted in SEQ ID NO: 2, expression vectors containing said nucleic acid molecule host cell comprising said vector, methods of producing said polypeptide,

3) Claims 17-25 (partially): An Asb-a polypeptide having an amino acid sequence with at least 39 % amino acid identity with SEQ ID NO: 3, nucleic acid thereof having at least 35% identity with a nucleotide sequence depicted in SEQ ID NO: 4, expression

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vectors containing said nucleic acid molecule host cell comprising said vector, methods of producing said polypeptide,

4) Claims 26-28: Stem cell or progenitor cell comprising an exogenous Asb-a polypeptide, an exogenous nucleotide sequence encoding an Asb-a polypeptide or both, pharmaceutical composition thereof.

The reasons for which the inventions are not so linked as to form a single general inventive concept, as required by Rule 13.1 PCT, are as follows:
Without taking into account the prior art, the common concept linking the abovementioned inventions could be seen in the Asb-a protein. However, the human Asb-a protein is already known from prior art (e.g. D1, D2). As the said common concept lacks novelty and there are no other features which could serve as special technical features according to rule 13.2 PCT, unity is lacking.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The current application concerns Asb-a proteins and their uses in expansion of stem cells. The Asb-a protein and nucleic acid from Zebrafish (seq id no 1 and 2) and its human homolog (seq id no 3 and 4) are disclosed. Asb-a is an ankyrin and SOCS box containing gene involved in the neuronal differentiation. Overexpression in PC12 cells leads to further division of the cells without differentiation. Claimed are the polypeptides, the corresponding nucleic acids and methods for in vitro expansion of mammalian stem or progenitor cells.

2. **Novelty** (Art. 33(2) PCT)

Claims 1-16, directed to methods using the Asb-a proteins for expansion of stem cells

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can be acknowledged novelty over prior art.

Similarly, claims 25-28, directed to stem cells or progenitor cells transfected with Asb-a are considered novel over the cited prior art.

3. **Inventive step** (Art. 33(3) PCT)

The product claims (and method to produce the polypeptide of the invention) 17-24, are neither novel nor inventive in view of the cited prior art:
D1 discloses the protein JT460914 which shows 49.8% identity over 263 aa with seq id no 1 and 100% identity over 329 aa (FL) with seq id no 3. AAI71054 shows 100% identity with SEQ ID NO 4 over 990 nt (full length).
Moreover, the Asb gene was functionally characterized in D2 and D3.
Therefore, the subject-matter of the product claims concerning the human or zebrafish Asb-a gene lack novelty or at least are immediately obvious over the cited documents D1 and D2 or D3.

Re Item VIII
Certain observations on the international application

1. Claims 1-16, 25-28 are considered to lack support and sufficiency of disclosure within the meaning of Art. 5 and 6 PCT. The effect of Asb-a on expansion of stem cells and progenitor cells has not been demonstrated in the documents as originally filed. All the experimental part is restricted to PC-12 cells, where overexpression lead to further division of the cells without differentiation. However, since PC-12 cells are derived from rat pheochromocytoma and are therefore neither stem cells nor progenitor cells. Thus, there is no obvious reason to assume that the effect of Asb-a on PC-12 cells can be generalized to all progenitor cells and stem cells as claimed.

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2. For the assessment of the present claims 1-17, 25-28 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The stem cells covered by the said claims include human stem cells (description p. 12, line 20). The use of human stem cells could be considered as offending morality.

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